Metabolic regulation of immunity in chronic HBV infection

Dr Laura Pallett
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Metabolic regulation of immunity in chronic HBV infection by amino acid deprivation

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Postdoctoral Research Associate – Prof. Mala Maini
Impact of Hepatitis B virus (HBV)

- Hepatotropic, non-cytopathic virus
- HBV establishes persistent liver infection in >300 million people worldwide
- Causes >600,000 deaths annually from associated liver disease
- Persistence is perpetuated by an inadequate virus-specific T cell response

Resultant liver disease is immune-mediated
Differential regulation of tissue damage in HBV infection

adapted from: Rehermann, Nat. Rev. Immunol. 2005
Differential regulation of tissue damage in HBV infection

Acute, resolving HBV

Chronic HBV

Adapted from: Rehermann, Nat. Rev. Immunol. 2005
Differential regulation of tissue damage in HBV infection

Paradigm for immunomodulation of organ damage - What mediates the differential regulation of liver immunopathology in different phases of HBV infection?
Innate regulation of adaptive immunity in HBV infection

Rapid, contact-dependent NK cell killing of HBV-specific T cells

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Up-regulation of a death receptor renders antiviral T cells susceptible to NK cell-mediated deletion

Dimitra Peppa

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Up-regulation of a death receptor renders antiviral T cells susceptible to NK cell-mediated deletion

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Role for myeloid-derived suppressor cells in regulating liver immunopathology

Metabolic regulation of hepatitis B immunopathology by myeloid-derived suppressor cells

Laura J Pallett, Upkar S Gill, Alberto Quaglia, Linda V Sinclair, Maria Jover-Cobos, Anna Schurich, Kasha P Singh, Niclas Thomas, Abhishek Das, Antony Chen, Giuseppe Fusai, Antonio Bertoletti, Doreen A Cantrell, Patrick T Kennedy, Nathan A Davies, Muzlifah Haniffa & Malu K Maini
What are granulocytic myeloid-derived suppressor cells?


Cancer
Trauma
Autoimmunity
(Bone marrow transplantation)

INFECTION

predominant population in humans

gMDSC
mMDSC
gMDSC expand in hepatotrophic viral infection (HBV)

11 colour flow cytometry - peripheral blood

all chronic HBV patients

gMDSC transiently expand in acute HBV in parallel with viraemia

Acute, resolving HBV

- Serum HBV DNA
- Serum ALT activity

Outcome:
- Clinical recovery

Incubation phase
- Acute disease, clinical symptoms
- Recovery, protective immunity

Graphs showing:
- serum ALT (IU/l)
- frequency of circulating gMDSC (as % of myeloid)
- HBV viral load (log10)
gMDSC transiently expand in acute HBV in parallel with viraemia

acute, resolving HBV

serum ALT (IU/l)
frequency of circulating gMDSC (as % of myeloid)
HBV viral load (IU/ml)

patient A1

HBV viral load (log10) vs. number of days

patient A2

HBV viral load (log10) vs. number of days

serum ALT (IU/l) vs. number of days

gMDSC (as % of myeloid) vs. number of days
...declining at the onset of liver-specific inflammation

acute, resolving HBV
gMDSC expand in patients replicating HBV without immunopathology
gMDSC expand in patients replicating HBV without immunopathology


gMDSC expand in patients replicating HBV without immunopathology

**biochemical**

- gMDSC (as a % of myeloid) vs. serum ALT (IU/L)
  - Correlation: $r = -0.29$
  - p-value: p = 0.040

**histological**

- gMDSC (as a % of myeloid) vs. necroinflammatory score
  - Correlation: $r = -0.49$
  - p-value: p = 0.0007

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**Graphs and charts**

- Increase (% of maximum) of gMDSC over time after infection (years)
- Serum HBV DNA
- Serum ALT activity

**Comparison**

- Immuno-tolerant
- eAg+ active disease
- Inactive disease
- eAg- active disease

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*p* values indicate significance levels:
- *: p < 0.05
- **: p < 0.01
gMDSC are increased in the absence of liver inflammation

<table>
<thead>
<tr>
<th>gMDSC (% of myeloid)</th>
<th>serum ALT (IU/L)</th>
<th>necroinflamm. score</th>
</tr>
</thead>
<tbody>
<tr>
<td>low</td>
<td></td>
<td></td>
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<tr>
<td>high</td>
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with thanks to Dr Niclas Thomas
gMDSC decline before the onset of hepatic flares in eAg- chronic HBV disease
gMDSC decline before the onset of hepatic flares in eAg- chronic HBV disease
*Ex vivo* data point to a role for gMDSC in suppressing liver inflammation

How could they achieve this?
Global metabolic defect in HBV T cells

- CD3ζ downregulation
- Dysregulation in functionality
gMDSC drive nutrient deprivation

Nutrient deprivation

- Loss of CD3 expression
- Proliferative arrest
- Altered protein translation

Tcell
- Activation of GCN2
- Inhibition of mTOR

- Depletion of L-arginine
- Depletion of L-cysteine
- Depletion of L-tryptophan

- Increased urea

- ARG1, NOS2
- IDO
  (sequestration of cystine)

- STAT3
- STAT6
- C/EBPb
- HIF1α
- MYD88

L-arginine
- Citrulline

伞状细胞 (gMDSC)
Proliferating T cells require extra amino acids as well as glucose
gMDSC produces arginase I which depletes L-arginine

Arginase I is increased and L-arginine is decreased in the serum

Arginase I quantitation: Serum ELISA

L-Arginine quantitation: tandem high-pressure liquid chromatography mass-spectrometry
Do gMDSC reach the liver, the site of HBV infection and pathology?
Do gMDSC reach the liver, the site of HBV infection and pathology?

Upkar Gill
Patrick Kennedy
QMUL: Barts & the London
gMDSC are further expanded in the intrahepatic compartment in HBV


IHL - intrahepatic leukocytes
** p = <0.01
*** p = <0.001 (Wilcoxon signed-rank test)
gMDSC are further expanded in the intrahepatic compartment in HBV

IHL - intrahepatic leukocytes
** p = <0.01
*** p = <0.001 (Wilcoxon signed-rank test)
What factors promote gMDSC expansion in the liver?
pHSC cells promote enhanced gMDSC proliferation/survival

thanks to K. Singh/ H. Singh/ E.S. Chambers: pHSC/skin fibroblasts isolation
Can arginase I+ gMDSC suppress T cell immunopathology in the liver?

- HBV is a non-cytopathic virus
- Liver damage: Initiated by HBV-specific CTL, amplified by bystander T cells

Maini et al, JEM 2000
Kakimi et al JEM 2001
Sitia et al, PNAS 2002
Can arginase I+ gMDSC suppress T cell immunopathology in the liver?

- HBV is a non-cytopathic virus
- Liver damage: Initiated by HBV-specific CTL, amplified by bystander T cells

Anatomic localisation of hepatic gMDSC?

*Visualisation of gMDSC in contact with T cells in liver vasculature*

CD3 red, CD66b brown

Immunohistochemistry: A. Quaglia
gMDSC potently suppress expansion of HBV-specific T cells
gMDSC suppress bystander T cells in a partially L-arginine dependent manner

0.5 μg/ml CEF peptide: CMV/EBA/Flu

**

% CD8+ IFN-γ

CEF  + gMDSC

% CD4+ IFN-γ

CEF  + gMDSC
gMDSC suppress bystander T cells in a partially L-arginine dependent manner

0.5 μg/ml CEF peptide: CMV/EBA/Flu

*CD8* IFN-γ^+ | *CD4* IFN-γ^+ | fold change over CEF stim
---|---|---
CEF | 6.00 | 1.00
CEF + gMDSC | 9.17 | 2.33
CEF | 1.50 | 1.00
CEF + gMDSC | 6.21 | 4.14
CEF | 2.50 | 1.00
CEF + gMDSC | 9.58 | 3.83
CEF + nonNOHA | 2.00 | 1.00
CEF + nonNOHA | 1.50 | 1.00
CEF + nonNOHA | 1.00 | 1.00
CEF + nonNOHA | 0.50 | 1.00
CEF + nonNOHA | 0.00 | 1.00
Ex vivo: T cells in HBV bare the hallmark of L-arginine deprivation acuity, resolving HBV

![Graphs showing CD3+ MFI and gMDSC frequency over time for patients A1, A2, and A3.]

- **CD3+ expression MFI on total T cells**
- **frequency of circulating gMDSC (as % of myeloid)**
Ex vivo: T cells in HBV bare the hallmark of L-arginine deprivation

acute, resolving HBV

chronic HBV

**patient A1**

**patient A2**

**patient A3**

**patient C1**

**patient C2**

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CD3ε expression MFI on total T cells

frequency of circulating gMDSC (as % of myeloid)
Metabolic regulation in HBV at the T cell level

Amino acid starvation impairs T cell proliferation & function impeding T cell responses that drive pathology

Do T cells initiate compensatory changes?
System-L amino acid transporters: critical checkpoint controlling T cell metabolism

Control of amino-acid transport by antigen receptors coordinates the metabolic reprogramming essential for T cell differentiation

Hypothesis: L-arginine starvation induces an up-regulation of system-L amino acid transporters on T cells
Compensatory increase in system-L amino acid transport in arginine-starved T cells

BCH: 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid
Intrahepatic and HBV-specific T cells have increased system-L amino acid transporter expression *ex vivo*

* IHL - intrahepatic leukocytes

PBMC IHL

CD98 MFI

0 200 400 600

***

PBMC IHL

CD98 MFI

0 200 400 600

***

global HBV+

CD98 MFI

0 200 400 600

***

* IHL - intrahepatic leukocytes

HBV-specific: HLA-A2-restricted multimer positive

Intrahepatic and HBV-specific T cells have increase system-L amino acid transporter expression \textit{ex vivo}

Amino acid transporters calibrate the T cell response to amino acid starvation

* IHL - intrahepatic leukocytes  
HBV-specific: HLA-A2-restricted multimer positive

Metabolic regulation of T cells in viral hepatitis

Amino acid starvation impairs T cell proliferation & function, impeding T cell responses that drive pathology.

Metabolically stressed T cells attempt reprogramming by compensatory increases in system-L amino acid transport.

A rheostat & potential therapeutic target to control immunopathology.
Summary

**gMDSC phenotype**
CD11b\(^{\text{high}}\)
CD33\(^{+}\)
HLA-DR\(^{-}\)
CD14\(^{-}\)
CD15\(^{+}\)
CXCR1
CXCR3
arginase I

**Peripheral blood**

**Liver**
arginase I degranulation (CD63)

**Metabolic reprogramming**
CD98
CD71
amino acid uptake

**Functional impairment**
- cytokine production
- proliferative capacity

**Bystander** (non-HBV-specific)
arginase I
low arg
Mala Maini
Abhishek Das
Antony Chen
Dimitra Peppa
Jessica Wijngaarden
Nick Easom
Anna Schurich
Kerstin Stegmann
Wei-chen Huang
Itziar Otano
Kasha Singh
Simran Singh

UCL
Nathan Davies
Maria Jover-Cobos
Richard Milne
Niclas Thomas
Eleni Nastouli
Emma Chambers

Kings College London
Oltin Pop
Alberto Quaglia

University of Dundee
Doreen Cantrell
Linda Sinclair

Royal Free Hospital
Kito Fusai
William Rosenberg
Francis Robertson
Prof. Davidson

Royal London Hospital
Patrick Kennedy
Upkar Gill

Agency of Science & Technology Research (A*STAR)
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